Phytopathol. Mediterr. (2000) 39, 404–409

Field susceptibility of four peach rootstocks to *Phytophthora citrophthora* and *P. syringae*

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Summary. Four two-year-old peach rootstocks were assessed for susceptibility to *Phytophthora citrophthora* and *P. syringae*. Peach rootstocks reacted differently to each *Phytophthora* species. GF677 rootstock was the less susceptible to *P. syringae*. The other rootstocks inoculated with this fungus did not differ significantly in the average necrosis length produced. Also peach rootstocks inoculated with *P. citrophthora* showed different level of susceptibility. Inoculation of GF677 plants with *P. citrophthora* produced the largest necrosis of all rootstocks tested. There were no significant differences in the lesion lengths of PR204, GF305, and KID I plants. The results from laboratory experiments with both fungi were similar to those from the field experiments: any observed differences can be attributed to differences in the physiology of the plant tissues and in the aggressiveness of the fungi.

Key words: crown rot, *Phytophthora* species, rootstocks, resistance, peach tree.

Introduction

Phytophthora crown and root rots are serious diseases of peach trees in Imathia County (Greece). Several species have been isolated from rot-infected trees including *Phytophthora syringae*, *P. cactorum*, *P. citrophthora*, and *P. megasperma* (Sarejanni, 1935; Kouyeas, 1971; Kouyeas 1977; Stylianidis *et al*., 1985; Chitzanidis and Stylianidis, 1987). Trees infected with *Phytophthora* usually show symptoms of apoplexy, of which there are two types in Greece. One occurs in summer and is usually caused by *P. cactorum* and *P. citrophthora*. Trees show sudden wilt, which is sometimes but not always preceded by mild leaf chlorosis. The bark and underlying cambium tissues, starting from the base of the tree and often extending several centimeters above soil level become damaged and gum exudation from the bark tissue is plentiful. The second type of apoplexy occurs in late winter or early spring and is due to *P. syringae* (Kouyeas, 1977). On young trees this type of apoplexy produces typical *Phytophthora* symptoms and the buds usually fail to open. Mature trees, in contrast, produce weak, stunted and chlorotic shoots. Finally, affected trees die in May or early June. Other species of *Phytophthora* have also been associated with crown rot of peach trees; these include *P. cinnamomi, P. cryptogea* and *P. cambivora* (Mircetich and Keil, 1970; Kim *et al*., 1985; Wilcox and Ellis, 1989). Peach rootstocks vary in their susceptibility to *Phytophthora* from region to region (Utkhede and Smith, 1994) and also among isolates of *Phytophthora* (Matheron and Matejka, 1990).

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Various methods have been developed to screen fruit trees for resistance to *Phytophthora* and to evaluate the virulence of *Phytophthora* isolates. Stem inoculation is a very common technique to screen rootstocks for crown rot resistance in the field (Kouyeas, 1971; Kouyeas, 1977; Stylianidis *et al*., 1985; Chitzanidis and Stylianidis, 1987; Lilja *et al*., 1996). The excised twig assay is used to determine the pathogenicity of isolates in the laboratory (Borecki and Millican, 1969; Jeffers *et al*., 1982; Flores and Hindal, 1983; Utkhede and Quamme, 1988; Scott *et al*., 1992; Tynan *et al*., 1998). This technique has also been used to measure seasonal variations in the extent of colonization by *Phytophthora* species (Jeffers and Aldwinckle, 1986; Browne and Mircetich, 1996). Excised stem inoculation has been used for determining the pathogenicity of pythiaceous fungi in the laboratory (Matheron and Mircetich, 1985; Matheron and Matejka, 1993). Both methods are simple, convenient and allow ample replications.

The purpose of this study was to compare four peach rootstocks (KID I, GF305, PR204, GF677) for susceptibility to crown rot.

Materials and methods

Phytophthora isolates

Two isolates of *P. syringae* (PSa and PSb) and two isolates of *P. citrophthora* (PCa and PCb) were used in all experiments. Both *P. citrophthora* isolates originated from citrus trees showing the typical *Phytophthora* gummosis, while the two *P. syringae* isolates were recovered from almond trees with typical *Phytophthora* crown rot. All isolates were kept in the Benaki Phytopathological collection at 22°C. Fresh cultures were obtained by transferring agar plugs to Petri plates containing cornmeal agar (CMA, DIFCO, Detroit, MC, USA). Cultures of *P. syringae* and *P. citrophthora* were grown at 16 and 23°C respectively.

Laboratory experiments

Excised twig assay

The excised twig assay, developed by Jeffers *et al*. (1981), was used to evaluate peach rootstocks for resistance to *P. citrophthora* and *P. syringae*. Seventy ml of CMA amended with antibiotics (pimaricin, 10 mg; ampicillin, 250 mg; rifampicin, 10 mg) was dispensed in jars to give an agar depth of about 10 mm. Eight jars per isolate were seeded with an agar plug of mycelium from a 7-day-old *Phytophthora* culture and sealed with parafilm to maintain a moist atmosphere. Two jars with each isolate were used to inoculate excised twigs of each rootstock. Two jars without inoculum were used as controls.

The jars were placed in chambers at a temperature appropriate for each species tested (23°C for *P. citrophthora* and 16°C for *P. syringae*) until colony growth nearly covered the agar surface. One-year-old woody shoots were collected in November and again in December. Segments 7 cm long and 1 cm in diameter were cut from the central part of the shoots and were disinfected in 10% domestic bleach (4.9%, sodium hypochloride). Segments were then rinsed in sterile water and blotted dry. The bark from the basal end of each twig was removed on opposite sides to expose the cambium. Ten of these pared twig segments were inserted vertically distal end up into the agar medium in each jar at the periphery of the fungal colony. The jars were resealed and incubated for six days at 16°C for *P. syringae* and for four days at 23°C for *P. citrophthora*. By subtracting depth of agar from the total length of necrosis, a value of necrosis length was obtained.

Excised shoot inoculations

Segments of woody shoots of each rootstock 6 cm in length and 1.5–2 cm in diameter were collected from 4-year-old mother plants in November and again in December 1999. The inoculum, consisting of a 6-mm diam plug from 5-day-old cultures of *P. citrophthora* and 10-day-old cultures of *P. syringae*, was inserted into the middle of segments under the bark. The wounds were covered with petroleum jelly and sealed with adhesive tape to prevent desiccation. Inoculated shoot segments were incubated for four days at 23°C for *P. citrophthora* and for six days at 16°C for *P. syringae*, in moist chambers, after which the length of the resulting lesions was recorded (Matheron and Mircetich, 1985).

Twenty segments from each rootstock were inoculated with each *Phytophthora* isolate. Segments inoculated with agar were used as controls.

Field experiments

Field experiments were conducted in the ex-

perimental field of the Pomology Institute, Naoussa (northern Greece) in 1998-1999. Seedlings of each rootstock were obtained from a commercial tissue culture station (Vitro Hellas, Alexandria, Greece) in 1996, and were planted directly in the field. Before and during the experiments, all essential cultural practices were carried out and the seedlings remained healthy.

Stem inoculation

The method was as described by Scott *et al*., (1992). Two-year-old plants were inoculated *in vivo* by raising a 6-mm-diameter piece of bark to expose the cambium and inserting a 6-mm-diameter plug taken from the margin of a *Phytophthora* culture growing on CMA, after which the bark was replaced and the wound sealed with adhesive tape. Inoculations with *P. syringae* were performed in November 1998 and repeated in March 1999, when temperatures favored the development of *P. syringae*; inoculations with *P. citrophthora* were made in May 1999 and again in September 1999 for the same reason. A month after inoculation, the adhesive tape was removed from each wound and the trunk bark scraped with a sharp knife to reveal any necrosis. The vertical length of the lesion was measured. Ten plants per rootstock were used for each *Phytophthora* isolate. Ten plants inoculated only with agar were used as controls.

To confirm that lesion development resulted from infection by *Phytophthora,* pieces from the margin of necrotic tissue were placed on the selective medium developed by Jeffers and Martin (1986) and incubated at 16°C for *P. syringae* and at 23°C for *P. citrophthora*.

Data analysis

The experimental design used in all experiments was completely random. The results were subjected to analysis of variance. Duncan's multiple range test was used to compare the resistance of peach rootstocks to *Phytophthora*. Each experiment was conducted twice.

Results

Laboratory experiments

Excised twig assay

Both *Phytophthora* species produced cankers on the excised twigs of all rootstocks. Cankers mainly developed upward. With PCa and PCb isolates KID I, GF305 and PR204 twigs developed canker of similar length after four days but on GF677 twigs canker length was more extensive (Table 1).

Discoloration was observed on the epidermis of twigs inoculated with both isolates of *P. syringae*, only some twigs showed gummosis. The development of PSa and PSb on KID I twigs was similar to that on GF305 and PR204 twigs but lesions on GF677 by these strains were significantly shorter. No necrosis developed on the control twigs (Table 1).

Excised stem inoculation.

Direct inoculation of *P. citrophthora* and *P. syringae* onto excised stems of peach rootstocks pro-

Table 1. Susceptibility to *Phytophthora citrophthora* and *P. syringae* as shown by canker length on four peach rootstocks tested by the excised twig assay.

Rootstock	Length of canker[a]			
	P. citrophthora[b]		*P. syringae*[b]	
	A	B	A	B
Control	0.00 c[c]	0.00 c	0.00 c	0.00 c
GF677	1.15 a	1.41 a	1.04 b	0.96 b
KID I	1.12 a	1.02 b	1.56 a	1.38 a
PR204	0.70 b	0.90 b	1.42 a	1.31 a
GF305	0.82 b	1.08 b	1.57 a	1.29 a

[a] Each value represents the mean of two experiments, each with twenty replicates.
[b] Two isolates (A and B) were used for each *Phytophthora* species.
[c] Values followed by different letters are significantly different (P=0.05) according to Duncan's multiple range test.

Table 2. Susceptibility to *Phytophthora citrophthora* and *P. syringae* as shown by canker length on four peach rootstocks tested by excised stem pieces.

Rootstock	Length of canker[a]			
	P. citrophthora[b]		*P. syringae*[b]	
	A	B	A	B
Control	0.00 c[c]	0.00 c	0.00 b	0.00 c
GF677	2.36 a	3.02 a	2.22 a	2.21 b
KID I	2.15 b	1.65 b	2.18 a	3.15 a
PR204	2.11 b	1.72 b	2.13 a	3.23 a
GF305	2.02 b	1.74 b	2.18 a	3.18 a

[a, b, c] See Table 1.

duced necrosis around the inoculation sites. In addition, gummosis was observed on all infected stem segments. In segments infected with *P. citrophthora* the typical necrosis extended upward and downward. Necrosis was most extensive in GF677 twigs. No significant differences in canker length were observed among KID I, PR204, and GF305.

Lesions on stem segments of KID I, PR204, and GF305 inoculated with PSa did not differ significantly. In contrast, colonization of GF677 segments by PSb was significantly less than that on other rootstocks. Symptoms with *P. syringae* were similar to those caused by *P. citrophthora*. No necrosis was observed on the control segments (Table 2).

Field experiments

Stem inoculation

Both *P. citrophthora* and *P. syringae* were recovered from two plants, which showed at least typical crown rot symptoms. No plants died during the trial.

Trees inoculated with *P. citrophthora* showed the typical crown rot symptoms and lost their leaves earlier than non-inoculated trees. Some trees also showed gummosis a week after inoculation. Colonization of GF677 by both isolates of *P. citrophthora* was the most extensive of all the rootstocks. There were no significant differences in canker length between PR204, GF305 and KID I rootstocks.

Resistance of peach rootstock to *P. syringae* was different from resistance to *P. citrophthora*. Externally, trees did not show crown rot or other symptoms, but removal of bark revealed necrosis. With *P. syringae* GF677 plants developed less necrosis than PR204 and KID I plants. However, there were no significant differences in the canker lengths produced in GF677, and GF305 by either isolates.

Table 3. Susceptibility to *Phytophthora citrophthora* and *P. syringae* as shown by canker length on four field grown peach rootstocks by the stem inoculation method.

Rootstock	Length of canker[a]			
	P. citrophthora		*P. syringae*	
	A[b]	B	A	B
Control	0.00 c	0.00 c	0.00 c	0.00 c
GF 677	3.70 a[c]	4.25 a	2.73 b	2.41 b
KID I	3.37 ab	3.58 b	2.92 ab	3.01 a
PR204	2.79 b	3.70 b	3.14 a	3.15 a
GF 305	2.76 b	3.63 b	2.99 ab	2.74 ab

[a] Each value represents the mean of two experiments, each with ten replicates.
[b, c] See Table 1.

There were also no significant differences in the canker lengths produced in KID I, PR204 and GF305 by either isolates. No necrosis was observed on the control plants (Table 3).

Discussion

In the presence study three techniques were used to evaluate the susceptibility of four peach rootstocks to *P. citrophthora* and *P. syringae*: stem inoculation *in situ*; *in vitro* inoculation of excised 1-year-old twigs and of 4-year-old shoots. To inoculate the plants or their sections, four isolates were used: two of *P. citrophthora* and two of *P. syringae*.

In general, although disease severity, as measured by length of necrosis at the sites of inoculation, varied between *in situ* and *in vitro* inoculation, no significant differences were observed among the techniques employed, nor among the reactions caused in the host tissues by each set of fungal isolates. Disease severity was higher with *in situ* inoculation of stems than with inoculation of excised shoots. Of *in vitro* inoculations, that on 1-year-old twigs caused a weaker reaction. These results are not surprising if it is considered that the reaction to inoculation of excised shoots may have been affected by changes in the physiology of excised tissues or plant parts. On the other hand, inoculation of standing plants only detected resistance mechanisms that were triggered after pathogen entrance.

With respect to fungal isolates, no differences were observed between the two isolates of *P. syringae* except in the case of inoculation of excised shoots; but isolate PCb of *P. citrophthora* appeared somewhat more virulent than isolate PCa, causing longer lesions on all four rootstocks, at least when the stem inoculation method was used. The average length of the stem cankers produced on GF677, KID I, PR204, and GF305 by the two pairs of isolates were 2.08 (PCa), 2.31 (PCb), 2.16 (PSa), and 2.33 (PSb).

None of the rootstocks were resistant to *P. citrophthora* or *P. syringae*. However, GF677 was more susceptible to *P. citrophthora* and less susceptible to *P. syringae* than the other three rootstocks, whose reaction to the two *Phytophthora* species did not differ significantly among each other. This last result was clear with standing plants. However, the overall average lenghts of stem cankers were 2.65, 2.15, 2.04, and 1.96 for GF677, KID I, PR204, and GF305 inoculated with *P. citrophthora* and 1.93, 2.37, 2.42 and 2.30 for the same rootstocks inoculated with *P. syringae*. These canker lengths clearly indicated that any differences in resistance were detectable in the experimental conditions used in this study. Additional study is needed to identify peach rootstocks with satisfactory resistance to *Phytophthora* crown and root rot.

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Accepted for publication: October 30, 2000